THE BIOLOGY AND BEHAVIOUR OF *PTINUS TECTUS* BOIE (COLEOPTERA, PTINIDAE), A PEST OF STORED PRODUCTS

V. HUMIDITY REACTIONS

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(With Eight Text-figures)

I. INTRODUCTION

This paper continues the series on *Ptinus tectus* (Bentley, Gunn & Ewer, 1941; Gunn & Hopf, 1942; Ewer & Ewer, 1942; Gunn & Walshe, 1942). The correlation to be observed between moisture conditions and the intensity of insect infestations of stored products needs no emphasis. For this reason it is desirable to analyse as far as possible the humidity reactions of such a well-established pest as *P. tectus* has become in this country. Although the species is not ideal for an attack on the outstanding problems of the humidity reactions of insects, the results obtained contribute to the growing body of knowledge in this field.

II. APPARATUS AND METHODS

The apparatus used in the majority of experiments was the *alternative chamber* or choice chamber of Gunn & Kennedy (1936) as modified by Bentley *et al.* (1941). This consists essentially of a shallow glass dish, 22 cm. in diameter, with a false floor of perforated zinc and a glass lid sealed by vaseline. Under the false floor are placed Petri dishes of the required strengths of sulphuric acid and the Edney paper hygrometers. In a few experiments a *ring gradient*, 90 cm. in diameter, was used (Gunn & Kennedy, 1936). In this, a circular track on perforated zinc is available to the animals, the gradient being controlled by granular calcium chloride and water and measured in the same way as before. All the work was done at a constant temperature of $25 \pm 0.5^{\circ}$ C. and under constant light from a high ceiling lamp which gave an approximate intensity of 20-30 m. candles at table level.

In the experiments on preferred humidity ten animals were used in each choice chamber. A zone, 1 cm. wide, divided the gradient into a drier part and a wetter part. After each reading the animals were moved by means of a pipe-cleaner into the middle of the dish, or so distributed that there were equal numbers on each side of the middle zone. A preliminary experiment showed that the maximum humidity reaction did not occur until 3 or 4 hr. after the animals had been dropped into the choice chamber; during this period they showed more activity than they did later. After this, maximal intensity of reaction occurred within 1 hr. of stirring. Accordingly, in all experiments the first observation was made $3\frac{1}{2}$ hr. after the animals had been put into the choice chamber and subsequent observations at hourly intervals. Using this method, eight readings were made in a day. For a number of reasons it was not found possible to follow the usual practice of repeating each experiment on the following day with the acid dishes and hygrometers reversed.

The index used to express intensity of reaction may be represented as $\{100 (D - W)\}/N$, where D represents the number of position records on the drier side, W the number of the wetter side and N the total number of observations (that is, including animals in the middle zone). The advantages of this index are that it gives equal prominence to 'wet' and 'dry' reactions and it takes into account those animals which were in the middle zone at the time of observation.

In the experiments on locomotory activity at different constant humidities, ten animals were used in each choice chamber. In recording, a distinction was made between itinerant or active beetles and beetles which were 'virtually inactive', that is, showing movements which, if continued, would not carry them to another part of the chamber (Pielou & Gunn, 1940). Observations were made by the cross-section method (Bentley *et al.* 1941) in which the observer chose a random instant at which to note the behaviour of the insects.

All animals were bred at 25° C. in constant illumination on wholemeal flour to which 5 % of dried baker's yeast had been added. A supply of water maintained a R.H. of about 70 % and also functioned as a drinking supply. Unless otherwise stated, beetles were removed from cultures on the day before an experiment and transferred to wet cotton-wool from which they could make up any water deficiency. After 15 min. a wad of dry cotton-wool was introduced. The beetles were picked off this on the following day and put in the experimental dishes. The dishes were set up on the previous afternoon and allowed to equilibrate overnight.

s at present the only way to obtain large numbers of *P. tectus* of the same sex is extremely laborious, the insects used were not chosen for sex. As far as possible beetles of approximately the same age were employed, ranging from 2 to 5 weeks old.

The experiments on the location of the receptors involved amputation of varying numbers of antennal segments. It was found unnecessary to anaesthetize. With practice it became possible to remove the required number of segments with a pair of iridectomy scissors after turning the beetle on its back and teasing with a fine paint brush until it gave

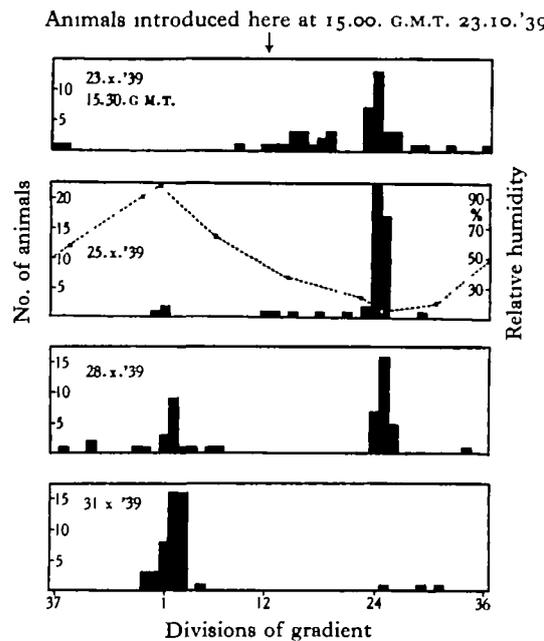Animals introduced here at 15.00. G.M.T. 23.10.'39

Fig. 1. Results from an experiment in the ring gradient. Fifty *Ptinus tectus* were watered and introduced as shown. After about a week the aggregation near the calcium chloride was replaced by a preference for wet air. The gradient remained steady throughout

a shamming reaction. Individuals which bled much were rejected, but they were very few in number. In these experiments the animals, together with their controls (which were subjected to approximately the same amount of handling), were kept in a damp atmosphere for 2 days before experiment.

III. RESULTS

1. *Experiments with the ring gradient*

To gain a rough idea of the nature of the humidity preference of *P. tectus*, fifty insects were allowed to make up any water deficiency and introduced into the ring gradient mentioned above. The apparatus gave a range extending from 14·5 to 98·0% R.H. representing a gradient of 0·6% R.H. per cm. It remained very constant throughout the experiment.

Fig. 1 shows that within 30 min. there was a con-

siderable aggregation in the dry region. This became more pronounced and then suffered little change for 5–6 days. The beetles forming the aggregation remained in a dense clump from which single animals would detach themselves temporarily, only to return after a few minutes. Towards the end of a week the insects began to disperse and to migrate up the gradient. After 8 days from the start of the experiment 94% of the beetles were in the immediate vicinity of the water. It was noticeable that the animals no longer clumped but formed much looser aggregations and were restless. At this stage one insect was dead. The remainder were removed from the gradient and separated into five groups. Each group was weighed, allowed to drink, dried and reweighed. The gain in weight was reasonably consistent, varying from 17 to 29% and averaging 22% of the desiccated weight. Most of the beetles were in air of about 16% R.H. for the greater part of the experiment, and the average rate of loss of water (approx. 2¼% per day) indicates considerable powers of resistance to desiccation (cf. 4% per day at 25°C and 0% R.H. in the case of *Tenebrio molitor* (Pielou & Gunn, 1940). In another similar experiment it was noticed that after the desiccated animals had been watered and put back in the gradient they usually, but not invariably, went *immediately* to the region of the calcium chloride as at the beginning of the experiment.

2. *The effect of humidity level and desiccation on locomotory activity*

The previous experiment indicated that the humidity preference of *P. tectus* depends largely on the degree to which the insect has lost water. Before proceeding to a more detailed analysis of the behaviour by means of choice-chamber tests, two sets of experiments were carried out to determine the effect of desiccation on locomotory activity in the absence of humidity gradients. The two sets yielded similar results, but the second only will be discussed.

Twelve lots of ten animals were watered and kept overnight at 70% R.H. The following day they were transferred to experimental chambers in which a constant humidity was maintained. The humidity levels chosen were 0, 30, 80 and 95%, and there were three experiments at each level. Eight hourly observations were made each day from 0930 to 1630 G M.T. Fig. 2 shows the mean activity (i.e. excluding virtual inactivity) for the three experiments at each humidity. Each point represents an average for two consecutive days (sixteen observations). For the first 12 days they are based therefore on 480 records. After this period some of the insects were dead. The total number remaining alive (out of thirty) at each humidity is shown on the graph.

Fig. 3 shows the 2-day averages for the individual experiments at 0% R.H., and indicates a fair degree of correspondence in the results obtained. It is clear that humidity level has a well-defined effect on loco-

motory activity. For the first week there is no significant difference between the activity at o and that at 30% R.H. At 80% R.H., however, the activity observed is about double what it is at the lower humidities, while at 95% R.H. it is approximately four times as high. Of particular interest is the behaviour of the insects at 0% R.H. and therefore
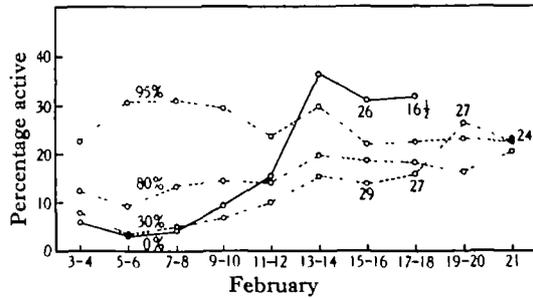

Fig. 2. Two-day averages from four sets of three experiments on the effect of humidity on the locomotory activity of *Ptinus tectus*. The values opposite certain of the points indicate the number of animals surviving out of thirty. Each of the other points represents 480 records.

subject to the greatest desiccation. At the end of a week the activity of these animals increased, till after 11-12 days it was greater than even that of the beetles kept at 95% R.H. A few days later nearly half had died and several of the remainder were obviously moribund. A similar increase in activity took place in the case of the insects at 30% R.H. but at a correspondingly later stage of the experiment.
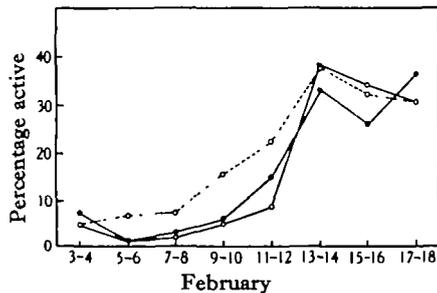

Fig. 3. Two-day averages of three experiments on the locomotory activity of ten *Ptinus tectus* at 0% R.H. Eight hourly observations were made each day.

These results are in agreement with those obtained in the experiments with the ring gradient. The low activity at first at 0% R.H. compared with that at 95% R.H. suggests that the aggregation at the dry end may be due to a simple kinetic mechanism. That the humidity behaviour of *P. tectus* is more complex than this is indicated by results of the choice-chamber experiments presently to be described. The increase of activity with desiccation is also consistent with the alteration of preference shown by the ring-gradient animals under similar conditions. The results

of both sets of experiments stress the importance humidity tests of choosing animals in which the degree of desiccation has been carefully controlled.

3. The humidity responses of watered beetles

Approximately 100 experiments were carried out in the choice chamber in an attempt to analyse the stages leading up to the final reaction observable in the ring gradient. The variation in the results obtained in preliminary experiments indicated the advisability of concentrating on small differences of humidity over six or seven different regions of the scale. Thus, in the majority of the experiments, the acid differences used were 0-15, 15-30, 30-45, 45-60,
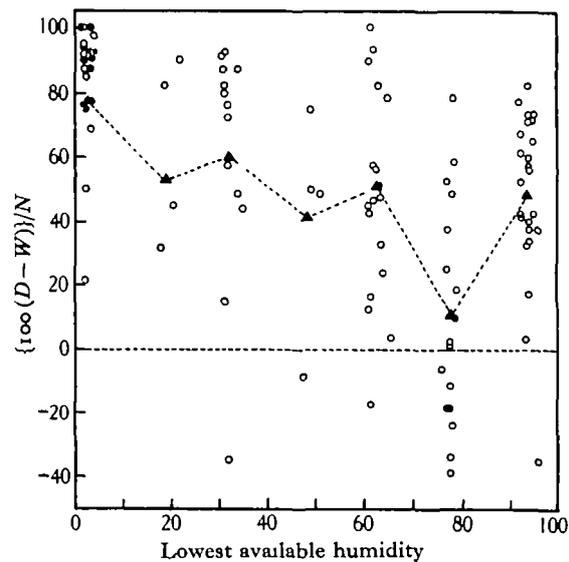

Fig. 4. The humidity preference of recently watered *Ptinus tectus*. Intensity of reaction is expressed in terms of the excess percentage of animals on the dry side and is plotted against the lowest humidity available. The ranges were 10·3-12·2% difference of humidity (5% at the 90% R.H. range), with the exception of ten experiments (indicated by blocked-in circles) in which the range available was approximately 18% R.H. The dotted line joins up the mean intensities of reaction.

60-75, 75-90 and 90-100% R.H. These gave actual humidity gradients of approximately 11% R.H. except at the wet end, where the average lay between 94 and 99% R.H. To avoid hysteresis effects in the hygrometers the calibration of the instruments used in dry air was never carried below 5% R.H., and the lowest humidities were therefore estimated by extrapolation.

Fig. 4 shows the values obtained for the mean intensity of reaction (in terms of excess percentage on the dry side of the gradient) in each of the experiments performed. Each point is derived from eighty position records extending over a period of 8 hr. Ten experiments were carried out with the steeper gradient obtained by using acids subtending atmo-

■eres of o and 25% R.H. These results are also indicated in Fig. 4. There were not enough of these experiments to show whether the lowest or highest humidity available is the more significant in determining the intensity of the reaction. In the present analysis the intensity is plotted against the lowest R.H. recorded.

The kind of variation in intensity of reaction to humidity revealed by *P. tectus* in different regions of the scale is unique when compared with the behaviour of all other animals studied. Ignoring the considerable individual variation for which no explanation is offered, it is plain that on the whole the insects show a preference for dry air. This preference is particularly intense both at the drier end of the gradient and in very damp air. In air in which there is a gradient between about 77 and 89% R.H. there is no significant reaction either one way or the other.

In the light of these results, whatever the underlying mechanism of the reaction, the aggregation at the dry end of the ring gradient might be expected. Insects at the wet end would tend to migrate into the region of no reaction. Any return to the wet end resulting from the appreciable activity already noticed at this R.H. level would not be permanent. On the other hand, a similar undirected wandering into a region below the zone of no reaction would tend to be continued towards the drier end. One might expect therefore to find a loose secondary aggregation in the 75–90% R.H. region in the early stages of the ring-gradient experiments. This has not been observed, possibly owing to the masking effect of the abnormally high activity which *Ptinus* invariably exhibits after handling.

The effect of steepness of gradient was not studied specifically, but the results of the above-mentioned experiments in the 0–25% R.H. range can be compared with those obtained from the ten experiments in the 0–15% R.H. range. In the first series the mean gradient used was 3·0–21·0% R.H. (a difference of 18·0% R.H.) and the mean I.R. was 92·0 ± 8·5%. The less steep gradient averaged 2·5–14% R.H. (a difference of 11·5% R.H.) and the mean I.R. was 77·7 ± 24·9%. Statistical analysis shows that the observed difference between these two means is insufficient to indicate a clear correlation between intensity of reaction and the steepness of the gradient offered.

4. The reactions of desiccated beetles

In view of the reversal of humidity preference which eventually occurred in the ring gradient, the experiments referred to in the previous section were repeated on a smaller scale with insects which had been kept without water for several days. Two sets of experiments were carried out using the following acid ranges: 0–15, 75–90 and 90–100% R.H. Desiccation was brought about by keeping the beetles for 10 days at 0% R.H. They may thus be compared with the insects used in the experiment on the effect

of 0% R.H. on activity level, at a time 10 days after the experiment had begun and when activity had become quite high (see Figs. 2, 3). They may also be compared with the ring-gradient insects after these had migrated to the wet end of the apparatus (Fig. 1). Two types of control were used. In the first series of experiments the control animals were desiccated with the experimental animals until a few hours before observations began and then they were given water. In the second series they were watered normally throughout the 10 days before the experi-
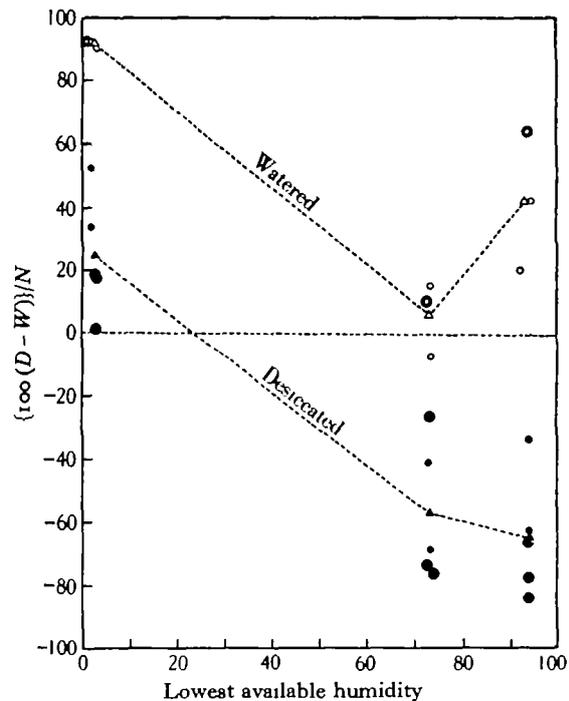

Fig. 5. Two sets of experiments on the humidity preference of desiccated *Ptinus tectus* in three gradients. The ordinates are the same as in Fig. 4. ○ controls, 1st set of experiments. ⊙ controls, 2nd set of experiments △ mean of both sets. ● desiccated beetles, 1st set of experiments. ⊙ desiccated beetles, 2nd set of experiments. ▲ mean of both sets.

ments. Fig. 5 shows the results obtained, each point on the graph representing, as before, eighty position records.

The two series of experiments gave essentially the same results. In each case the values obtained for the controls compare well with the mean intensities of reaction established in the experiments described in the previous section (Fig. 4). It may be inferred that, whatever the changes in the nature of the humidity response which occurred in the desiccated insects, they were reversed on drinking. It is justifiable, therefore, to combine the results from the two sets of control experiments. This has been done in Fig. 5.

The desiccated beetles behaved very differently from beetles which had recently drank. In the 0–15% R.H. range the reaction was still apparently towards the drier side though reduced in intensity. In the other two ranges the 'dry' reaction was replaced by a reaction towards the wetter end of the gradient. This result is not inconsistent with the migration to the 'wet' zone shown by the ring-gradient insects after desiccation, for, whereas the slight 'dry' reaction in the lowest humidity range would have a trapping effect, this would be offset by the enormous increase in activity which occurs with desiccation (Fig. 2). Such an increase of activity might easily take the insect outside the range where the 'dry' response is effective.
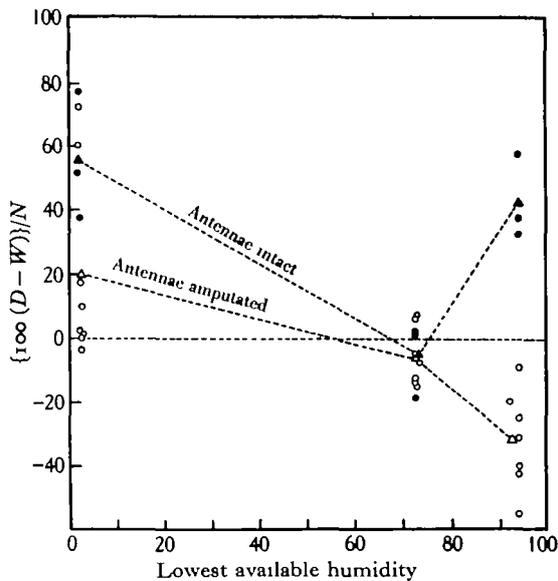

Fig. 6. The humidity preference of antennae-less *Ptinus tectus* in the same gradients as were used in the experiments shown in Fig. 5. Blocked-in symbols indicate control experiments, triangles indicate the mean intensities.

5. Experiments on the location of the humidity receptors

Possible interpretations of the reversed reaction to humidity shown by *Ptinus* when it is desiccated may depend on the nature of the receptors. An attempt was therefore made to locate them. The information obtained, though incomplete, may be of interest as far as it goes. The antenna of *P. tectus* consists of eleven segments, of which the basal and the most distal are rather larger than the other nine. In the experiments to be mentioned, extirpations of various numbers of segments were carried out. The operations were always bilateral and in no case was the basal segment removed as it was considered inadvisable to operate too near the brain. Preliminary experiments showed that, though the removal of segments apparently did not affect the

proportion of the day during which the anim█ moved about, the operated beetles moved at a mu█ slower speed than their controls. As reaction to humidity and activity level are usually closely linked it is therefore inadvisable to draw comparisons between strengths of response of de-antennate and antennate insects. Fig. 6 shows the results of a number of choice-chamber experiments on watered beetles in which ten of the eleven segments of the antenna had been removed. The three humidity ranges used were the same as in the experiments on
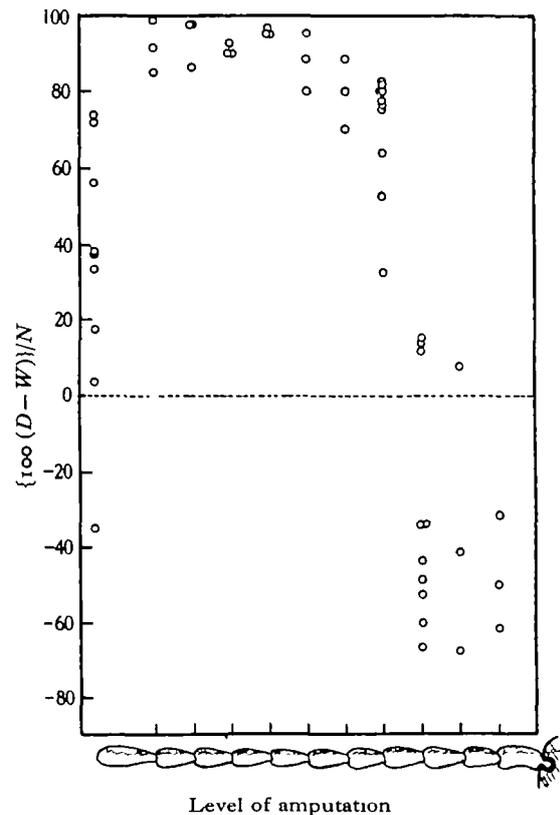

Fig. 7. The humidity preference of *Ptinus tectus* between 94 and 99½% R.H. following bilateral extirpation of antennae at various levels. The control experiments are shown to the left.

desiccated animals. In the 0–15% R.H. range the preference was still 'dry' but less than that shown by the controls. In the 75–90% R.H. range there was no significant reaction. In very damp air, however, the operated beetles behaved very much like unoperated, *desiccated* beetles, giving a strong 'wet' reaction.

This result led to some fifty further experiments in the 90–100% R.H. range with beetles lacking various numbers of antennal segments. Fig. 7 shows the results obtained. When the terminal segment was removed an increased preference for dry air occurred. This persisted with successive extirpations

six segments had been removed. Thereafter the density of the dry reaction fell off rapidly until with only three segments remaining the reaction was predominantly towards the wetter air. Interpretation of the results of these two sets of experiments will require further experiment, especially in view of the reduced, but significantly, dry reaction shown by antenna-less beetles in the 0–15% R.H. gradient. They do suggest, however, the possibility of two sets of receptors being involved, of which one set is mainly confined to the distal eight segments of the
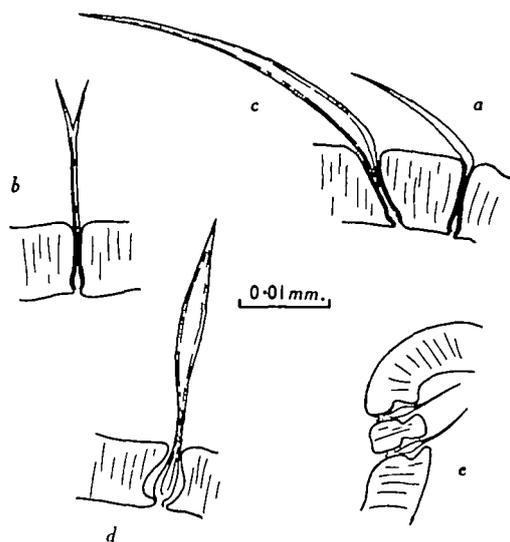

Fig. 8. Structures found on the antennae of *Ptinus tectus*. (a) short bristles abundant on distal segments; (b) forked bristles most abundant on proximal segments and on head; (c) coarser bristles also more frequent on proximal segments; (d) spear-shaped bristles occurring in large numbers only on terminal segment; (e) pit-hairs from rounded knob on which basal segment is set

antenna. Examination of sections and whole mounts failed to reveal among the various structures present any which either suggested a humidity-receptive function or which corresponded in distribution to that suggested above (Fig. 8). The antenna of *P. tectus* bears, for example, nothing resembling the humidity receptors described by Valentine (1931) and identified by Pielou (1940) for *Tenebrio molitor*, sections of the antenna of this insect being cut by the same technique for the purpose of comparison. Nor are there any structures on the *antenna* resembling the humidity receptors identified for the louse, *Pediculus humanus*, by Wigglesworth (1941).

IV. DISCUSSION

In spite of the considerable variation in intensity of response to humidity, when *Ptinus tectus* has had access to water to drink, it generally aggregates in the driest region of a gradient. The tendency is not, however, equally apparent over all humidity ranges

and is usually absent between 75 and 90% R.H. No explanation for this latter fact is offered. There is no reason to expect that an insect's receptors are equally sensitive over all ranges of stimulation, more particularly in this case where it is difficult to see what advantages would accrue from being able to differentiate between regions of a gradient which is probably equally favourable over its whole extent.

The experiments described above also show that as desiccation proceeds the reaction towards drier air disappears and is replaced by a reaction towards regions of higher humidity. Furthermore, the change in direction of response is accompanied at approximately the same point in time by a considerable increase of activity. The biological advantages of this are obvious.

Reactions towards dry air have been recorded for other insects. Kennedy (1937) noted a small but definite dry reaction over the whole of the scale in his experiments on the gregarious phase of *Locusta migratoria*. Here the intensity of reaction depended on the difference of humidity available. When this was less than 20% R.H. the reaction approached zero.

Less marked dry reactions were also found by Thomson (1938) in *Culex fatigans*, Gunn & Cosway (1938) in *Blatta orientalis*, and Pielou & Gunn (1940) in *Tenebrio molitor*. As pointed out by Kennedy, all these reactions result in aggregation in air the humidity of which might be considered as unfavourable. *Locusta* develops best between 60 and 75% R.H. (Hamilton, 1936); *Ptinus tectus* breeds best at about 70% R.H. (Ewer & Ewer, 1942); Thomson (1938) states that hungry females of *Culex fatigans* do not avoid humidities that are fatal. It would seem then that this preference for dry air invites comment. It must be remembered, however, that in the field the air humidity may never be as unfavourably low as it is in these experiments. Further, even when low humidities occur, it probably happens that other physical agencies combine to prevent the dry reaction taking place. As shown by Bentley *et al.* (1941), *Ptinus tectus* is most active at night. Temperature is then lowest and humidity highest for the day. The beetle's photonegative behaviour would also tend to keep it in a sheltered and therefore probably damper environment during hours of sunlight and high temperature.

The reversal of direction of response on desiccation is an added protection against prolonged exposure to dry atmospheres. Gunn & Cosway (1938) in their studies of the cockroach have also recorded a slight wet reaction after desiccation. The point may be raised as to whether *Ptinus* is often able to obtain liquid water in the field or whether it maintains its water balance by utilizing the water content of its food. Observations on the distribution of the insect in warehouses and stores in this country, as distinct from its original natural environment, suggest that the latter may usually be the case and that it lives permanently on the verge of desiccation.

From the economic viewpoint this may be fortunate, for Ewer & Ewer (1942) have reported a rise in the rate of oviposition in laboratory beetles which had been given water to drink. Their results are supported by the observation occasionally made that *P. tectus* shows a higher incidence in situations where food has become damaged by water, though it is true that this may be related to other factors such as the optimal humidity for larval development. In any case it is suggested that the humidity reactions of beetles found as pests of stored products in this country may be less markedly towards drier air than those of watered insects bred in the laboratory. Experiments begun with a view to deciding this point were unfinished when the work had to be suspended.

V. SUMMARY

1. *Ptinus tectus* adults react to a humidity gradient by collecting in the drier region.

2. The reaction to a given difference of humidity is most intense at low humidities; it declines to nothing at about 75–90 % R.H. and increases again at 90–100 % R.H. No such variation has previously been found.

3. A kinetic mechanism of reaction is involved. The higher the humidity the higher the locomotory activity over a period of a week.

4. Desiccated animals are more active than normal ones. At low humidities they show a much weakened reaction towards drier air; at high humidities the normal reaction is reversed and the animals collect in the wetter region.

5. Desiccated animals given water to drink immediately begin to behave like normal ones.

6. Some of the humidity receptors appear to be located on the antennae. Removal of the distal joint increases the intensity of reaction in high humidities, but the interpretation of this is questionable. The reaction remains strong when up to five joints are amputated and then it declines. When only one or more of the last three joints remain, a reversed reaction occurs, similar to that shown by desiccated individuals.

7. This suggests that there are two kinds of humidity receptors, the first kind being mainly located distally on the antennae and some of the second kind being either on the basal joint or not on the antennae at all.

8. Attempts to identify humidity receptors were not successful.

In conclusion, the writer would like to express his appreciation of the help and advice rendered by Dr D. L. Gunn and the encouragement given by Prof. H. Munro Fox, F.R.S., and Prof. Lancelot Hogben, F.R.S., during the course of the work.

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